IN THE UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

UNITED FARM WORKERS OF AMERICA, AFL-CIO; NATURAL RESOURCES DEFENSE COUNCIL; THE BREAST CANCER FUND; CALPIRG; CHARITABLE TRUST; PESTICIDE WATCH EDUCATION FUND; PESTICIDE ACTION NETWORK; SAN FRANCISCO BAY AREA PHYSICIANS FOR SOCIAL RESPONSIBILITY; MARCIA CUMMINGS HUBBARD; EUGENE D. HUBBARD, Petitioners,	)	
v.	)	Case No. 99-71143
CAROL M. BROWNER, ADMINISTRATOR, U.S. ENVIRONMENTAL PROTECTION AGENCY Respondent.	)	

DECLARATION OF ANTHONY F. MACIOROWSKI

I, Anthony F. Maciorowski, declare under penalty of perjury as follows:

1. I am a Senior Scientist in the Office of Science Coordination and Policy, Office of Prevention, Pesticides, and Toxic Substances, of the U.S. Environmental Protection Agency (EPA or the Agency). In this position, my responsibilities include overseeing implementation of EPA's Endocrine Disruptor Screening Program. The Endocrine Disruptor Screening Program (the Program) is the program that EPA developed to comply with section 408(p) of the Federal Food Drug and Cosmetics Act, as amended by the Food Quality Protection Act of 1996.

2. EPA's implementation of the Endocrine Disruptor Screening Program currently is proceeding on several fronts: EPA is in the process of completing the Endocrine Disruptor Priority Setting Database and the compartment-based approach that the Agency will use to establish priorities for actual screening later in the Program. EPA also is in the process of ensuring that the Tier 1 and Tier 2[1] assays that are part of the Program are validated as required

by statute.

Priority Setting

3. EPA is in the process of completing development of a compartment-based approach for setting priorities for evaluating chemicals under the Endocrine Disruptor Screening Program. Compartment-based priority setting involves the identification of information that can be used for comparing chemicals and the grouping of chemicals sharing this information into sets or compartments. Because all chemicals in a compartment share the same information, they can be compared and ranked. Such information might include environmental release, receptor binding, and levels and/or frequency that a chemical has been found in environmental media.

4. As part of this compartment based approach, EPA is developing a relational data base known as the Endocrine Disruptor Priority Setting Data Base (EDPSD). The EDPSD will contain information on the chemicals, sort them into compartments, and prioritize them within the compartments. EPA is currently compiling data and information on exposure and effects of chemicals to complete the development of the data base.

5. As part of the priority setting process, EPA currently is conducting Quantitative Structure Activity Relationship (QSAR) analysis on most pesticide active ingredients, pesticide formulation ingredients, and selected commodity chemicals. In the case of endocrine disruptors, the QSAR model predicts the ability of a chemical to bind with estrogen and androgen receptors. Estrogen and androgen receptors are proteins found in cells in many parts of the body. Natural hormones circulating in the body bind with these receptors and may activate or block various biological functions (e.g., growth, sexual development, etc.) associated with the hormone. The process is analogous to a key (the hormone) fitting into a lock (the receptor) and unlocking or locking a door (activation or deactivation of the biological activity). Chemicals that bind with the

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[1]The Tier 1 and Tier 2 assays are discussed generally later in this affidavit; they are described in detail in EPA's Endocrine Distruptor Screening Program; Proposed Statement of Policy (63 Fed. Reg. 71542, 71550-58 (Dec. 28, 1998)).

receptor may therefore alter or block natural hormone function, and such alteration or blocking is the first step in causing certain types of adverse effects. QSAR analysis relies on computer simulation to quantitatively link the activity of a chemical with certain features associated with its structure. QSAR prediction is based on a comparison of the fit of the molecular structure of the chemical into the molecular structure of the estrogen and androgen receptors. Therefore, QSAR analysis provides EPA with initial estimates of the potential for a chemical to interfere with the human endocrine system. EPA expects to complete its initial QSAR analysis by January 31, 2000.

Validation

6. EPA laboratories and contractors are currently validating a number of assays included in the program. Because certain assays are of international interest, EPA is also working with the Organisation for Economic Co-operation and Development (OECD) to standardize and validate several other assays included in the program. All of the EPA validation work is being conducted in close liaison with the Interagency Coordinating Committee for the Validation of Alternative Methods established by the National Toxicology Program, under the auspices of the National Institute of Environmental Health Sciences.

7. Validation is a scientific process that consists of: developing a method for conducting a scientific assay; evaluating the method's relevance (its ability to meet its stated purpose); and ensuring the method's reliability and repeatability among different laboratories. For endocrine disruptors, the specific methods being validated are the Tier 1 and Tier 2 assays specified in EPA's Endocrine Disruptor Screening Program (63 Fed Reg 71542, 71550-58, Dec. 28, 1998). EPA's Endocrine Disruptor Screening Program validation process consists of several general stages: 1) assay development; 2) demonstration of the relevance and reliability of an assay leading to a standardized protocol;[2] 3) a validation study to determine the inter-laboratory transferability of a standardized protocol and the comparability of results obtained at different

[2]A protocol is the detailed, step by step instructions for conducting an assay.

laboratories; and 4) independent scientific peer review of the validation study results.

8. EPA is actively working toward validation of the following Tier 1 assays: a uterotrophic assay; a Hershberger assay; a rodent pubertal female assay; a rodent pubertal male assay; estrogen and androgen receptor reporter gene assays; a fish reproduction assay, and a frog metamorphosis assay. EPA is also actively working toward validation of the following Tier 2 assays: a two generation mammalian reproduction and development assay and a mysid shrimp reproduction and development assay. In addition, EPA has convened and participated in meetings and workshops regarding the development of initial protocols for a mammalian developmental screening assay and an avian reproduction assay.

9. **The Uterotrophic Assay.** The rodent uterotrophic assay is designed to screen chemicals for estrogenic activity. It detects the ability of a chemical to stimulate or inhibit estrogenic responses of the uterus. The OECD, with participation from EPA, is in the process of developing the assay and standardizing its protocol (Steps 1 and 2 of the process described in paragraph 7). Preliminary findings suggest that the assay is robust and ready for protocol standardization and inter-laboratory validation.

10. **The Hershberger Assay.** The Hershberger assay is designed to screen chemicals for androgenic activity. It detects the ability of a chemical to stimulate or inhibit androgenic responses in testes and secondary sex organs. The OECD, with participation from EPA, has completed the development of the rodent Hershberger protocol (Step 1 of the process described in paragraph 7), and is ready to initiate a study leading toward a standardized protocol (Step 2 of the process described in paragraph 7). EPA scientists are leading the coordination of this study.

11. **The Rodent Pubertal Female Assay.** The rodent pubertal female assay is designed to screen for estrogenic and thyroid activity in immature female animals exposed to chemicals as they go undergo sexual maturation. The assay examines abnormalities associated with development of the female sex organs and secondary sexual characteristics. The Agency is in the process of standardizing the operational protocol for the rodent pubertal female assay (Step 2 of the process described in paragraph 7). An EPA contractor is conducting this assay using six

endocrine active chemicals and two different strains of laboratory rats.

12. **The Rodent Pubertal Male Assay.** The rodent pubertal male assay is designed to screen for androgenic and thyroid activity in immature male animals exposed to chemicals as they undergo sexual maturation. The assay examines abnormalities associated with development of the male sex organs and secondary sexual characteristics. The Agency is in the process of standardizing the operational protocol for the rodent pubertal male assay (Step 2 of the process described in paragraph 7). An EPA contractor is conducting this assay using six endocrine active chemicals and two different strains of laboratory rats.

13. **Fish Reproduction Assay.** The fish reproduction assay is designed to screen chemicals for estrogenic and androgenic effects. The assay examines abnormalities associated with survival, reproductive behavior, secondary sex characteristics, and fecundity (number of spawns, number of eggs per spawn, fertility, and development of offspring). The fish reproduction assay has undergone development and demonstration of an operational protocol (Steps 1 and 2 of the process described in paragraph 7). EPA has conducted the assay with four endocrine active chemicals and prepared a report of the study results.

14. **The Frog Metamorphosis Assay.** The frog metamorphosis assay is designed to screen chemicals for thyroid effects. Metamorphosis is under thyroid control and is a surrogate for screening potential thyroid effects in humans. The assay examines abnormalities associated with the tail resorption of tadpoles as they metamorphose into frogs. The frog metamorphosis assay has undergone assay development and demonstration of an operational protocol (Steps 1 and 2 of the process described in paragraph 7). EPA has awarded a contract to conduct the assay with four endocrine active chemicals, has received the data and has prepared an initial draft report of the results.

15. **Estrogen and Androgen Receptor Reporter Gene Assays and Other *In Vitro* Assays**. Estrogen and Androgen Receptor Reporter Gene Assays are designed to detect the consequences of binding to the estrogen or androgen receptor (activation or deactivation of a biological process or blocking of the receptor). Reporter gene assays provide actual

measurements as compared with computer simulated estimates provided by QSAR analysis described in paragraph 5. EPA concluded an High Throughput Prescreening (HTPS) demonstration study of several reporter gene assays in March 1999. HTPS is high-speed, automated technology that the pharmaceutical industry has used extensively in new drug research. EPA initiated the endocrine disruptor HTPS demonstration study to determine whether the technology was valid for conducting reporter gene assays on a large number of chemicals in a very short time frame. The HTPS demonstration study (Steps 1 and 2 of the process described in paragraph 7) revealed that the selected assays evaluated were insufficiently robust to be reliably used in routine screening applications without additional development. Therefore, the Agency has drafted a request for proposals to demonstrate the feasibility of using different *in vitro* technologies for routine screening of endocrine disruptors. In the interim, EPA has initiated methods development research (Step 1 of the process described in paragraph 7) on both estrogen receptor and androgen receptor reporter gene assays, a DNA membrane method for screening thyroid activity, and has initiated a review of DNA array technology to evaluate its potential for use in endocrine disruptor screening.

16. **Mysid Shrimp Reproduction Assay.** The mysid shrimp assay is designed to characterize dose-response characteristics and adverse reproductive and developmental effects of chemicals. EPA developed and successfully demonstrated the use of a protocol for a two-generation Tier 2 assay for the mysid shrimp (Steps 1 and 2 of the process described in paragraph 7).

17. **Mammalian Reproduction Assay.** The EPA/OPPTS revised Test Guideline on Reproduction and Fertility Effects (OPPTS 870.3800, August 1998) is designed to characterize dose-response characteristics and adverse reproductive and developmental effects of chemicals. The OPPTS 870.3800 test guideline contains new and important endpoints for estrogenic and androgenic effects. In addition, EPA is currently updating the guideline to add important thyroid effects. This new protocol will be demonstrated in two studies to begin in early 2000.

I declare under penalty of perjury that the aforementioned is true and correct, to the best

of my knowledge and belief.

Executed on December 21, 1999, in Washington, D.C.

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Anthony F. Maciorowski, Ph.D.
Senior Scientist